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Precocious puberty in a boy with a PRL-, LH- and FSH-secreting pituitary tumour: Hormonal and immunocytochemical studies.

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Abstract. The case of a 7-year-old boy affected with precocious puberty and a large intra- and suprasellar pituitary tumour is described. He had hyperprolactinemia and elevated serum LH, FSH and testosterone concentrations. Pre-operative dynamic hormonal studies showed a rise of PRL, LH and FSH levels after TRH (200 µg iv) and a rise of LH and FSH after GnRH (100 µg iv). Dopamine infusion (4 µg · kg⁻¹ · min⁻¹ for 180 min) did not affect gonadotropins and greatly reduced serum PRL. GnRH analogue (buserelin, 0.5 mg sc t.i.d. for 10 days) administration inhibited both LH and FSH, but did not affect PRL concentration. Serum LH and FSH increased after ethinyl-estradiol (0.5 mg orally) administration, and were not affected by bromocriptine (5-7.5 mg/day for 10 days), which decreased serum PRL levels. The patient underwent transfrontal neurosurgery and a large tumour mass was completely removed. Morphological study of the excised tumour, by electron microscope double label immunotechnique, revealed that a large number of tumour cells (70-85%) were positive for PRL, LH and FSH, co-localized in the same secretory granule. After neurosurgery, serum PRL, LH, FSH and testosterone levels fell to within the normal limits. Two months later the patient was well and signs of precocious puberty had partially regressed; hormone levels were in the normal range and MR imaging control did not demonstrate any residual lesion in the sellar region.

True precocious puberty in boys is an uncommon disorder. When an organic cause is identifiable, the most common ones are hypothalamic lesions (craniopharyngioma, astrocytoma, hamartoma) or pineal tumours (1). Pituitary tumours rarely occur

in childhood and adolescence and they are usually associated with delayed onset of puberty and hypogonadism (2); these features have been particularly described in young patients with prolactinomas (3) and in one girl with LH- and PRL-secreting pituitary tumour (4). So far, precocious puberty in patients with pituitary adenomas has been described, to our knowledge, in only two patients, one with a prolactinoma (5) and one with a LH- and PRL-producing tumour (6).

In this paper we describe a 7-year-old boy with precocious puberty and a large pituitary tumour who had hyperprolactinemia and slightly elevated LH and FSH levels. Pre- and postoperative hormonal investigations and morphological studies by electron microscope double-label immunotechnique on the excised tumour are reported.

Case report

A boy aged 7 years and 4 months was referred to our hospital for precocious puberty and the presence of a large intra- and suprasellar lesion at CT scan. On physical examination, his height was 115 cm (10th percentile) and his weight 26 kg (75th percentile). Neither gynecomastia nor galactorrhea was present. Isosexual advanced puberty was revealed by the presence of testes of 4.5 ml volume, penis of 9.5 cm length and pubic and axillary hairs; the Tanner stage was P2 G3. X-ray examination showed a bone age of 9 years. Visual field perimetries demonstrated left temporal hemianopsia and visual

acuity was 2/10 in the right eye and 2/50 in the left one. Nuclear magnetic resonance (MR) showed a sellar and suprasellar lesion; about two-thirds of the mass had an intense signal in T_1 -weighted images, and a less intense one in T_2 , a finding compatible with hemorrhage or adipose tissue (Fig. 1).

Routine laboratory tests were normal. Endocrinological evaluation revealed high serum PRL, LH, FSH and testosterone basal levels (see Results).

The patient underwent short-term treatments with a GnRH analogue (buserelin, 0.5 mg t.i.d. sc for 10 days), which was not followed by any clinical modifications, and then with bromocriptine (5-7.5 mg/day for 10 days). On bromocriptine therapy visual field perimetries improved, temporal hemianopsia disappeared, and visual acuity was 8/10 in both eyes. The MR imaging control showed only a slight reduction of the tumoural lesion.

Twenty-four h after the last bromocriptine dose the patient underwent transfrontal neurosurgery and a large tumoural mass was completely excised.

Postoperative course was uncomplicated and visual fields defect was completely restored. Soon after neurosurgery serum PRL, LH, FSH and testosterone levels fell to within normal limits for age and sex. Two months later

the patient was well and signs of precocious puberty had partially regressed; hormone levels remained in the normal range and MR imaging control did not demonstrate any residual lesion in the sellar region (Fig. 1).

Hormone analyses

The concentrations of LH, FSH, alpha-subunit, PRL, testosterone, TSH, GH, ACTH, cortisol and free thyroid hormones were assessed in basal conditions and after the following tests, as appropriate: GnRH (100 µg iv), TRH (200 µg iv), ethinyl-estradiol (0.5 mg orally in a single dose), D-ser-(TBU)6-Pro-9-Net LHRH (buserelin, Hoechst, Italy, 0.5 mg sc t.i.d. for 10 days), dopamine infusion, ($4 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 180 min), bromocriptine (5-7.5 mg/day for 10 days), somatostatin analogue (SMS 201-995, octreotide, Sandoz, Switzerland, 50 µg sc), GHRH (1 µg/kg iv, Bachem, Switzerland), CRH (1 µg/kg iv, Novabiochem, Switzerland).

Serum alpha-subunit concentrations were measured by a previously reported RIA method (7), using reagents kindly provided by National Pituitary Agency, NIDDK, (Bethesda, MD). The lowest measurable alpha-subunit concentration was 0.2 µg/l. The intra- and inter-assay coefficients of variation were 4.1 and 5.8%, respectively.

The cross-reactions of TSH (MRC 6: 78/549) in the alpha-subunit RIA were respectively, whereas for LH (MRC 6 on a molar basis, the cross-reaction high alpha-subunit concentrations, and in the presence of high levels of

Serum LH, FSH and TSH levels immunoradiometric assays (Sucrose tech. UK), which show very high sensitivity. LH and FSH, 0.07 mU/l (for TSH) a particular, alpha-subunit, even at very (1000 µg/l), did not cross-react in the assays. Commercial kits were used for PRL, testosterone (Biodata, Italy), C-mones, ACTH, and cortisol.

Light microscopy

Pieces of tumour were fixed in 10% and embedded in paraffin. Sections hematoxylin-eosin.

Electron microscopy and immunocytochemical techniques

Small cubes of the surgically removed fixed at 4°C in 2% glutaraldehyde in buffer, pH 7.4, containing 1% sucrose, OsO_4 in cacodylate buffer at 4°C, bedded in Epon 812. Ultrathin sections uranyl acetate and lead citrate and tips CM10 electron microscope (Philips, Eindhoven, The Netherlands).

Protein A-gold complexes of different sizes were prepared as described by Slot & Geuze. Immunostaining was performed as previously. Sections were mounted on coverslips and treated with ethanol saturated with 10% H_2O_2 in order to remove endogenous peroxidase. The antisera used were an anti-hPRL (G. Friesen, Winnipeg, Manitoba, Canada), affinity chromatography, an anti-hFSH β serum (both obtained from the National Pituitary Agency Distribution Program). PRL and FSH were immunolocalized using slides of different sizes according to (10) and Geuze et al. (11). Incubation at room temperature for 2 h (antisera and A-gold complexes). Sections were then treated with 1% glutaraldehyde. The specificity of the immunostaining was tested by substituting normal rabbit serum for the antisera: 1. substituting normal rabbit serum for the antisera; 2. substituting normal rabbit serum with its respective antigen; 3. substituting normal rabbit serum for the antisera; 4. using different steps of the reaction. To determine the composition of the adenoma, 20 from 3 random tissue blocks were analysed by electron microscopy.



Fig. 1. Nuclear magnetic resonance (MR) images showing the presence of a sellar and suprasellar lesion in the patient before treatment (left panel) and its disappearance after neurosurgery (right panel).

d signs of precocious puberty had hormone levels remained in the imaging control did not demon- on in the sellar region (Fig. 1).

LH, FSH, alpha-subunit, PRL, ACTH, cortisol and free thyroid d in basal conditions and after the copriate: GnRH (100 µg iv), TRH radiol (0.5 mg orally in a single -Pro-9-Ner LHRH (buserelin, 7 se (1 d, for 10 days), dopamine, nin⁻¹ for 180 min), bromocriptine lays), somatostatin analogue (SMS Sandoz, Switzerland, 50 µg sc), chem, Switzerland), CRH (1 µg/kg zerland).
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parasellar lesion in the patient before

The cross-reactions of TSH (MRC 68/38) and FSH (MRC 78/549) in the alpha-subunit RIA were 3.7 and 1.6%, respectively, whereas for LH (MRC 68/40), as determined on a molar basis, the cross-reaction was absent even at high alpha-subunit concentrations, both in the absence and in the presence of high levels of the specific antigen. Serum LH, FSH and TSH levels were determined by immunoradiometric assays (Sucrosep IRMA, Boots-Celltech, UK), which show very high sensitivity (0.3 U/l for LH and FSH, 0.07 mU/l for TSH) and specificity; in particular, alpha-subunit, even at very high concentrations (1000 µg/l), did not cross-react in the above IRMA methods. Commercial kits were used for determination of PRL, testosterone (Biodata, Italy), GH, free thyroid hormones, ACTH, and cortisol.

Light microscopy

Pieces of tumour were fixed in 10% buffered formaline and embedded in paraffin. Sections were stained with hematoxylin-eosin.

Electron microscopy and immunocytochemical techniques

Small cubes of the surgically removed adenoma were fixed at 4°C in 2% glutaraldehyde in 0.12 mol/l cacodylate buffer, pH 7.4, containing 1% sucrose, postfixed in 1% (xO₂) in cacodylate buffer at 4°C, dehydrated, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips CM10 electron microscope (Philips Industries, Eindhoven, The Netherlands).

Protein A-gold complexes of different sizes were prepared as described by Slot & Geuze (8). Double immunostaining was performed as previously reported (9). Ultrathin sections were mounted on nickel grids with Formvar and treated with ethanol saturated with NaOH and then with 10% H₂O₂ in order to remove the epoxy resin. The antisera used were an anti-hPRL serum (gift of Dr H. G. Friesen, Winnipeg, Manitoba, Canada), purified by affinity chromatography, an anti-hLHβ serum, and an anti-hFSHβ serum (both obtained from NIDDK Hormone Distribution Program). PRL and LH or PRL and FSH were immunolocalized using protein A-gold particles of different sizes according to Bendayan & Zollinger (10) and Geuze et al. (11). Incubations were carried out at room temperature for 2 h (antisera) and 60 min (protein A-gold complexes). Sections were stained with uranyl acetate. The specificity of the immunostaining was tested by 1. substituting normal rabbit serum for anti-LHβ, anti-FSHβ and/or anti-PRL serum; 2. absorbing each antiserum with its respective antigen; 3. omitting one component of the reaction, and 4. using the antiserum in both steps of the reaction. To determine the percent cellular composition of the adenoma, 200-300 cells in sections from 3 random tissue blocks were examined by electron microscopy.

Results

Endocrinological evaluation revealed that the basal concentrations were above the normal limits for age and sex for serum PRL (mean 376 µg/l; range 300-450; normal values <16µg/l), LH (mean 3.8 IU/l; range 2.3-6.3; normal values <2 IU/l), FSH (mean 1.9 IU/l; range 0.6-4.1; normal values <1.5 IU/l) and testosterone (mean 20.6 nmol/l; range 16-25.3; normal values <4 nmol/l). Alpha-subunit levels were 0.64 µg/l (range 0.3-1.0; normal limits <0.7 µg/l) and β-hCG levels were not detectable. GnRH test induced a marked LH response up to 23.5 IU/l and a normal FSH rise up to 3.9 IU/l. TRH caused an abnormal LH rise to 8.3 IU/l, whereas FSH increased up to 2.6 IU/l (Fig. 2); serum PRL slightly rose from 380 to 430 µg/l. Alpha-subunit concentration rose from 0.3 to 2.2 µg/l after GnRH and from 0.4 to 1.1 µg/l after TRH (not shown).

Dopamine infusion did not significantly affect serum FSH and LH, whereas it reduced PRL levels

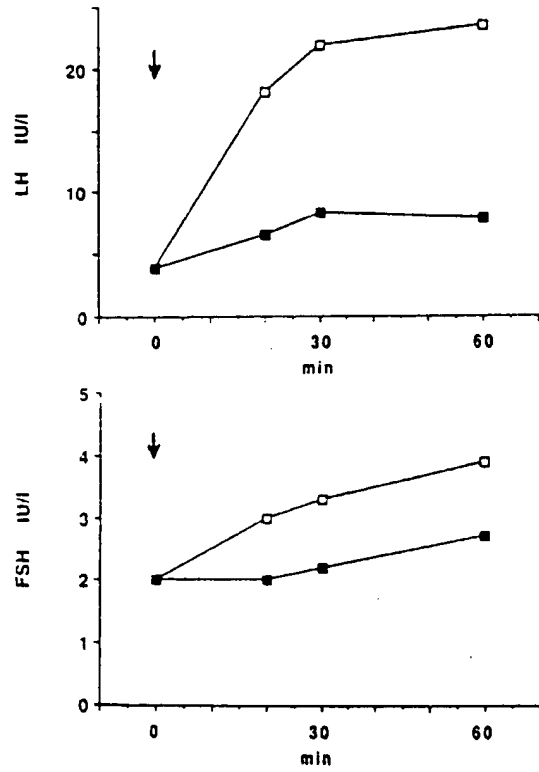


Fig. 2. Responses of serum LH and FSH after 200 µg TRH (■) and 100 µg GnRH (□) iv.

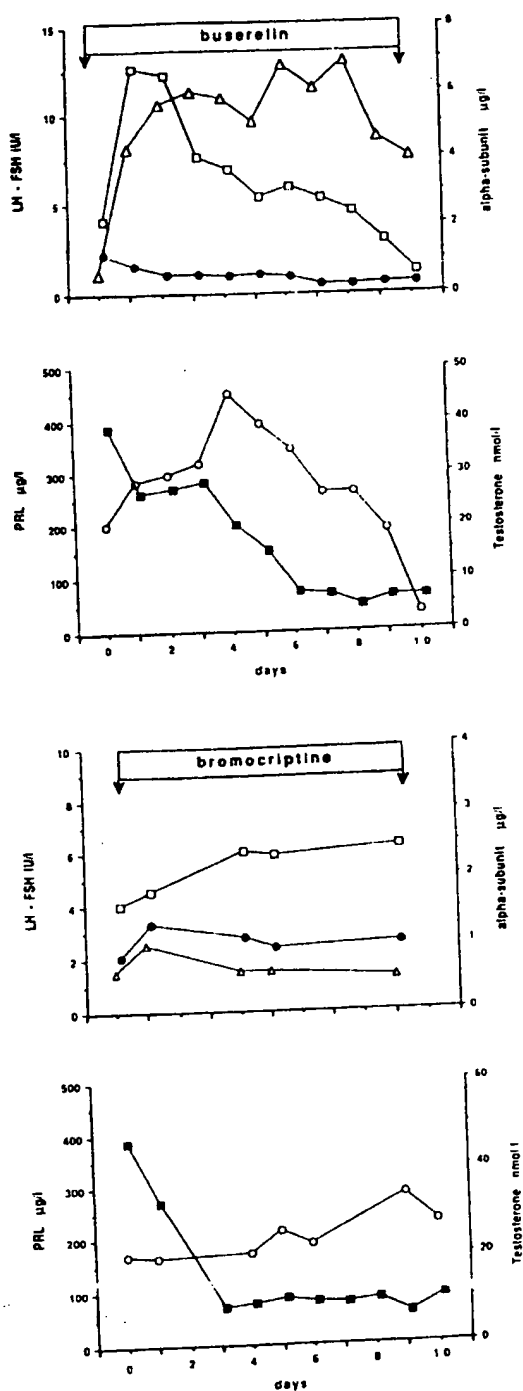


Fig. 3. Effects of GnRH analogue (buserelin, 0.5 mg sc t.i.d. for 10 days; top panel) and bromocriptine administration (5-7.5 mg/day for 10 days; bottom panel) on serum LH (\square - \square), FSH (\bullet - \bullet), alpha-subunit (Δ - Δ), PRL (\blacksquare - \blacksquare), and testosterone (\circ - \circ) levels.

from 300 to 110 $\mu\text{g/l}$. Injection of the somatostatin analogue (octreotide) did not modify serum LH, FSH and PRL levels (data not shown).

Administration of 0.5 mg ethinyl-estradiol did not cause any inhibition of LH, FSH and alpha-subunit, but rather an elevation of serum FSH (from 1.4 to 3.4 IU/l after 4 h) and LH levels (from 2.5 to 4.1 IU/l at 6 h); alpha-subunit slightly increased from 0.7 to 0.9 $\mu\text{g/l}$ at 6 h.

During GnRH analogue administration (Fig. 3) serum FSH levels were rapidly reduced and remained constantly low, whereas serum LH and testosterone, after an initial increase, progressively fell. During this treatment the secretory pattern of alpha-subunit completely dissociated from that of intact gonadotropins, as a progressive increase in alpha-subunit levels was observed. A decrease in PRL levels was observed. Bromocriptine treatment (Fig. 3) did not significantly affect serum LH, FSH, alpha-subunit and testosterone levels, which remained above the normal limits, whereas serum PRL was concomitantly reduced.

A normal responsiveness of GH, ACTH and cortisol, TSH and free thyroid hormones after GHRH, CRH and TRH tests, respectively, was found. Urinary 17-KS excretion was at the upper limit for age and sex ($=13.2 \mu\text{mol}/24 \text{ h}$; normal limits $<12.5 \mu\text{mol}/24 \text{ h}$).

After transfrontal adenectomy the excised tumour was studied. By histology the adenoma was acidophil and monomorph. Electron microscopy revealed that the adenoma was made up by large, oval or oblong cells, similar to each other. The nuclei were round, the rough endoplasmic reticulum appeared strikingly deformed with enlarged cisternae. The secretory granules were small, numerous and, in some cells, peripherally located (Fig. 4). Double-gold immunolabelling with anti-PRL and anti-LH β sera showed that the great majority (80-85%) of the cells was positive for PRL and LH β (Fig. 4, panel A). The two hormones were co-localized in the same secretory granule. The remaining cells were positive only for PRL. With anti-PRL and anti-FSH β sera many cells (about 70%) were positive for both hormones (Fig. 4, panel B), demonstrating that most cells contained both LH and FSH together with PRL.

After neurosurgery serum PRL, LH, FSH and testosterone levels fell to within normal limits. On the 12th day a combined multiple test with TRH, GnRH, CRH and GHRH induced a normal response of pituitary hormones, except for PRL

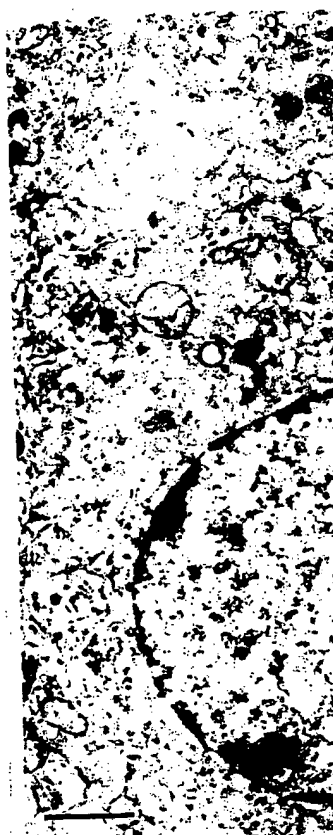


Fig. 4. Electron micrograph of portions of an adenoma. Many, small secretory granules are visible (cisternae scattered throughout the cytoplasm and protein A-gold particle particles) and LH (large gold particles) and FSH (small gold particles) are co-localized in the same secretory granule. B: Coexistence of LH and FSH in the same secretory granule (bar = 0.1 μm). About 70%

which did not react to the stimulus. Normal free thyroid hormone concentrations and normal 17 urinary excretion were also observed.

Discussion

Gonadotropin-secreting pituitary adenomas are uncommon - 3-7% of all pituitary tumours. They mostly occur in adult males and are associated with precocious puberty. Also prolactinomas are associated with gonadal dysfunction in adolescent patients (3). On the other hand, the appearance of precocious puberty

/l. Injection of the somatostatin le) did not modify serum LH, s (data not shown). f 0.5 mg ethinyl-estradiol did ition of LH, FSH and alpha r an elevation of serum FSH l after 4 h) and LH levels (from i h): alpha-subunit slightly in, 0.9 µg/l at 6 h.

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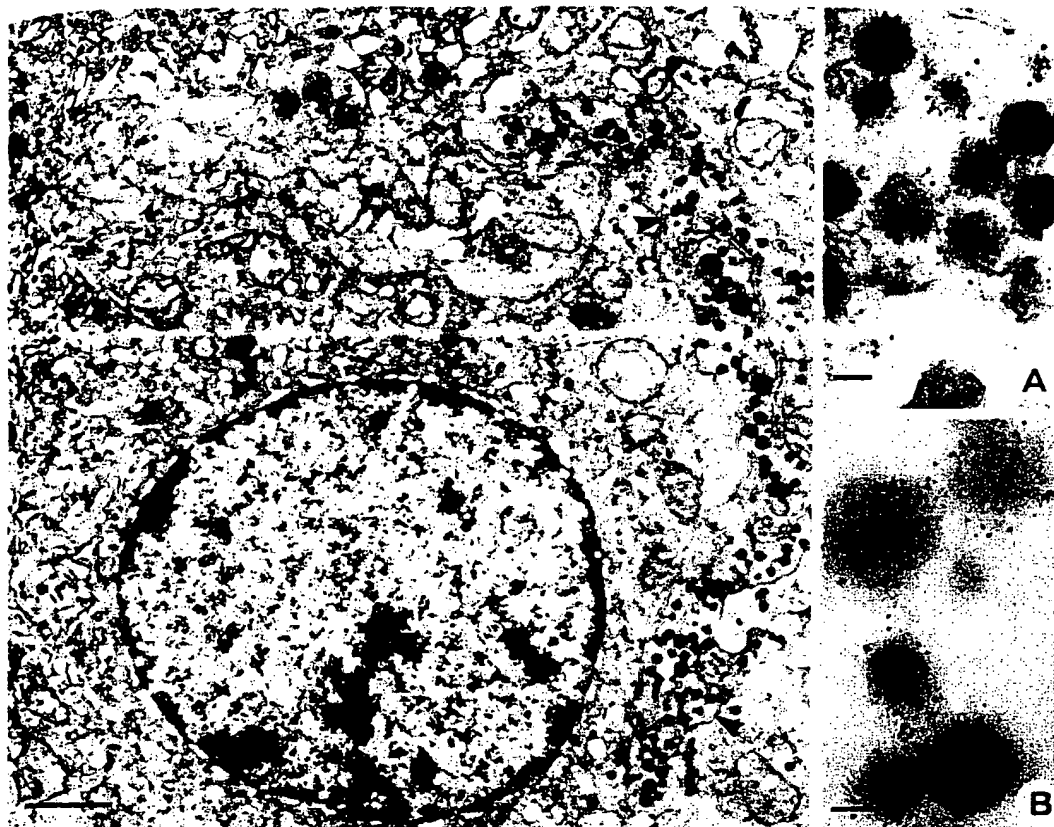


Fig. 4. Electron micrograph of portions of cells from the monomorphous pituitary adenoma secreting PRL, FSH and LH. Many, small secretory granules are visible (arrow head). Rough endoplasmic reticulum appears constituted by enlarged cisternae scattered throughout the cells (bar = 1 µ). Double immunolocalization of PRL/LH and PRL/FSH using specific antisera and protein A-gold particles of different sizes is shown in the lateral panels. A: Coexistence of PRL (small gold particles) and LH (large gold particles) in the same secretory granules (bar = 0.1 µ); 80-85% of the cells were positive for the two hormones. B: Coexistence of PRL (small gold particles) and FSH (large gold particles) in the same secretory granules (bar = 0.1 µ). About 70% of the cells were positive for the two hormones.

which did not react to the stimulus. In basal conditions normal free thyroid hormones and cortisol concentrations and normal 17-OHCS and 17-KS urinary excretion were also observed.

Discussion

Gonadotropin-secreting pituitary adenomas are uncommon - 3-7% of all pituitary tumours (12-14); they mostly occur in adult males and cause hypogonadism. Also prolactinomas are related with deficiency of gonadal function in both adults (15) and adolescent patients (3). On the contrary, the appearance of precocious puberty has been reported,

to our knowledge, in only two patients with pituitary tumours, a boy with an LH- and PRL-producing tumour (6) and another boy with a prolactinoma (5). In the present case an unusual concomitant hypersecretion of LH, FSH and PRL was caused by a pituitary adenoma and was the most likely explanation for the onset of precocious puberty. The patient actually had serum basal LH and FSH levels above the normal range for his age and sex; a marked LH rise with a mild FSH and alpha-subunit increase was elicited by GnRH, thus confirming the asserted existence of variable and often dissociated patterns of response of LH and FSH after GnRH in patients with gonadotropinomas (12,16).

The lack of influence of dopamine infusion on LH and FSH levels may possibly account for the negligible effect of chronic bromocriptine administration on gonadotropins, indicating the value of the test in predicting the response to chronic dopaminergic treatment (17). The effectiveness of bromocriptine therapy in patients with gonadotropin-secreting pituitary tumours is uncertain; in fact FSH and alpha-subunit levels can be reduced (17-19), but an evident tumour shrinkage has not been observed (20,21).

Besides variable responsiveness to physiological agents, an abnormal hormonal reaction to a non-specific releasing hormone was observed in this patient. An abnormal response to TRH has been found in about 80% of tested patients with gonadotropinomas (16,19). Although an increase in both gonadotropins is frequently induced by TRH in patients with only LH- or FSH-secreting tumours (16,19), a dissociation between the abnormal rise of the hypersecreted tropin and the absent response of the normally circulating gonadotropin has also been reported (16). In our patient a significant increase in LH was caused by the tripeptide. The somatostatin analogue, which has been reported to cause inhibition of GH, TSH, PRL and ACTH release from normal and/or tumoural cells (22), did not affect gonadotropin secretion.

After ethinyl-estradiol administration a lack of inhibition, but rather a rise of LH, FSH and alpha-subunit levels was observed in the present patient, at variance with the normal gonadotropin suppression observed in normal subjects. Variable patterns of responses to different estrogens, given for various periods of time, have been reported. A paradoxical elevation of the hypersecreted hormone and alpha-subunit in one patient (23) and of alpha-subunit levels in another (18) has been already described, thus suggesting a possible usefulness of the test in patients with gonadotropinomas.

Although an abnormal increase in gonadotropin levels during GnRH analogue treatment has been observed in few patients with gonadotropin-secreting adenoma (24,25), in the present case the GnRH analogue buserelin was able to lower LH and FSH levels, probably through a down-regulation mechanism, similar to that observed in normal subjects. The progressive increase in alpha-subunit levels during GnRH analogue treatment, as observed in girls with idiopathic precocious puberty (26), indicates a different control of alpha- and beta-subunit synthesis by GnRH (27).

In addition to gonadotropin hypersecretion, this patient showed a high serum PRL concentration, which exhibited a complex pattern of responses, probably related to the different cell types sustaining hyperprolactinemia. In fact, the adenoma showed a sensitivity to dopaminergic inhibition, in terms of reduction of PRL secretion and mild shrinkage of tumour mass, in spite of persistently high gonadotropin levels. This finding might be explained by the possibility that dopaminergic drugs preferentially affect pure lactotropes, which in part constituted the adenoma. Although it has been recently shown that a GnRH agonist can reduce PRL secretion by a paracrine action (28), the observed decrease in serum PRL during buserelin administration might be explained by an effect of the analogue on the cells co-secreting gonadotropins and PRL.

As far as the morphological findings are concerned, the present study confirms that in human pituitary adenomas cells producing more than one hormone are frequently present. In fact, the sensitive electron microscope protein A-gold immunotechnique, which detects very small amounts of hormonal antigens and identifies the simultaneous presence of two antigens in the same tissue secretion, now permits to recognize a large number of mixed cells in normal and pathological conditions (7,9,29,30). In this adenoma the so far unreported association of PRL, LH and FSH in the same cell is clearly demonstrated and was the cause of the precocious puberty of the boy; in fact the removal of the tumour led to a normalization of the endocrine function and to remission of the disease.

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gonadotropin hypersecretion, this high serum PRL concentration, complex pattern of responses, the different cell types sustaining the adenoma. In fact, the adenoma is resistant to dopaminergic inhibition, in spite of PRL secretion and mild tumor mass, in spite of persistently high levels. This finding might be the possibility that dopaminergic receptors affect pure lactotropes, which is the adenoma. Although it has been shown that a GnRH agonist can act by a paracrine action (29), the decrease in serum PRL during hypogonadism might be explained by an action on the cells co-secreting gonadotropin.

Morphological findings are consistent with the study confirms that in human pituitary cells producing more than one hormone are present. In fact, the immunohistochemical and pathological conditions of the adenoma the so far unreported co-secretion of LH and FSH in the same cell is demonstrated and was the cause of the hypersecretion in the boy; in fact the removal of the adenoma normalized the endocrine secretion and the disease.

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Sex steroids modula

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Abstract. The sex-related dif the regulation of TSH secreti igated by determination of t clear and TRH membrane re activity of 5'-deiodinase (5'D) itary gland of adult male and spective modulatory roles of grns on these parameters wer determinations carried out in trogen-treated male rats. Th T_3 and TRH receptors and t ll were significantly greater the male rats. The E_2 -treated female profile, viz. also great TRH receptors when compa rats (2.3 ± 0.2 vs 1.8 ± 0.2 f 9.4 ± 0.8 vs 6.0 ± 0.8 fmol \pm SEM), whereas no changes r treated rats. The E_2 -treated rats exhibited an increased p type II (0.87 ± 0.10 and 0.6 vs control 0.34 ± 0.07 p protein) $^{-1}$), suggestive of a s and of an inhibitory effect parameter. In contrast, no 5'D were found between all well-known tissue-specific re results demonstrate that the density of pituitary T_3 and l activity of 5'D in the adult modulatory effect of estrog sponsible for the sex-depend secretion.

It is well established that th dysfunctions is greater in w

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